A 1H-MR Spectroscopy Study of Changes in Glutamate and Glutamine (Glx) Concentrations in Frontal Spectra after Administration of Memantine

Glutamate is the major excitatory neurotransmitter in the brain and therefore important for cognitive functions. The aim of the study was to investigate if administration of the N-methyl-D-aspartate receptor antagonist memantine to healthy individuals would affect brain activation when performing an auditory attention task. The task was a variant of a dichotic listening task with different instructions that tap demands for attention and cognitive control. We asked the question if memantine administration would lead to a reduction in glutamatergic neurotransmission in areas related to attention and cognitive control. Left and right frontal glutamate and glutamine (Glx) concentrations were measured, using 1H-MR spectroscopy. Twenty-five healthy adults were scanned twice in a counterbalanced design, either drug naïve or after administration of memantine for 21 days. The results showed that memantine significantly reduced Glx concentrations, and this reduction was associated with a reduction in brain activation in prefrontal cortex, which could have implications for understanding the neuronal mechanisms underlying higher cognitive functions such as cognitive control.

Keywords: cognitive control, fMRI, glutamate and glutamine, 1H-MR spectroscopy, memantine

Introduction

Glutamate is the primary excitatory neurotransmitter in the brain and therefore glutamate-mediated synaptic transmission is critical for brain functions (Cooper et al. 2002). The N-methyl-D-aspartate (NMDA) receptor, which has a high affinity for glutamate is also widely distributed throughout the brain. It has been shown that the glutamatergic system is involved in excitatory synaptic transmission, plasticity, and excitotoxicity in the central nervous system (Cull-Candy et al. 2001). Moreover, signal transmission malfunction at the NMDA receptor site has been implicated in several neurological and psychiatric disorders such as Alzheimer’s disease and schizophrenia (Beal 1995; Olney et al. 1999) and that this may be caused by altered glutamate concentration. Previous studies attempting to modulate the NMDA receptor functioning by administration of NMDA antagonists like ketamine have also shown that the glutamatergic system is important for normal cognitive functions, such as memory, attention, and verbal fluency (Krystal et al. 1994; Malhotra et al. 1996; Newcomer et al. 1999). Memantine, which is used for treatment of Alzheimer’s disease, is an uncompetitive (e.g., does not bind to the agonist site of the NMDA receptor), open-channel blocker that exerts its effect on NMDA receptor activity by binding near or at the Mg2+ site within the ion channel (Lipton 1993, 2004) with low affinity for the channel pore. It has been shown that memantine blocks prolonged influx of calcium that occurs at pathological activation of NMDA receptors while relatively sparing normal neurotransmission (Danzysz et al. 2000). Memantine has been demonstrated to be well tolerated due to the favorable kinetics, inducing minimal adverse effects at doses within therapeutic range (20 mg/day; Lipton 2004). A few studies have focused on the neuronal effects of memantine in healthy adults (Schulz et al. 1996; Schugens et al. 1997; Rammsayer 2001; Korostenskaja et al. 2007). The results from these studies suggest that a single dose of memantine could affect cognition in healthy human subjects and that it can lower the participants’ arousal level.

In a recent study, we demonstrated that memantine reduced neuronal activation in the prefrontal cortex (PFC) extending to the anterior cingulate gyrus (ACC) in a dichotic listening task that engages cognitive control functions (van Wageningen et al. 2009; see also Asbjørnsen and Hugdahl 1995; Hugdahl et al. 2009). This suggests that memantine modulates neuronal activation in brain regions involved in cognitive control functions, in turn implicating the role of the glutamatergic neurotransmitter system in cognitive control regulation. However, there were no significant differences between the drug-naïve versus memantine sessions on behavioral performance, when they performed the dichotic listening task (van Wageningen et al. 2009). Several pharmacological fMRI studies using the NMDA receptor antagonist ketamine have shown changes in brain networks that subserve the cognitive process that was explored without corresponding changes in behavioral performance (Deakin et al. 2008).

The term cognitive control has been described as the cognitive processes that allow us to guide thought and behavior in accordance with our internal goals. Thus, cognitive control is the formation, maintenance, and realization of internal goals and is essential to cope with cognitive challenges in everyday life (Miller and Cohen 2001). Cognitive control is invoked by environmental stimuli that are perceptually salient or that trigger default action tendencies (Braver and Cohen 2000; Miller and Cohen 2001; Braver et al. 2002). Cognitive control can be experimentally induced by using the forced-attention dichotic listening paradigm with different instructions that tap demands for attention and cognitive control (Hugdahl et al. 2003, 2009). This paradigm consists of series of pair-wise presentations of simple syllable sounds with a different syllable presented in the right and left ear on each trial. The subject has to verbally report which of the 2 sounds they perceive on each trial (not being informed that there are 2 different sounds). The general result is that subjects tend to report more often those syllables presented to the right ear which constitute a bottom-up, or stimulus-driven, effect caused by left hemisphere lateralization of speech perception. This has
been labeled the right ear advantage (REA). By explicitly instructing the subjects to focus attention to either the right or left ear syllable, the REA can be modulated, and it is possible to study a top-down modulation of a bottom-up laterality effect, in the same experiment. When participants are instructed to focus attention on and report only the syllable from the right ear (forced right, FR), the REA is significantly increased, whereas when instructed to only report the syllable from the left ear (forced left, FL), REA is reduced or shifted to a left ear advantage, that is, more reports from the left ear (Hugdahl and Andersson 1986). The FR and FL conditions both involve top-down attention instruction modulation, however, it is possible to induce an attentional conflict situation when the subject is instructed to attend to and report the left ear stimulus. This situation requires attentional control in the form of suppression of the bottom-up stimulus-driven effect of reporting the right ear stimulus (due to left hemisphere specialization for speech sound perception). Because the bottom-up component drives the right ear perceptual response, whereas the top-down component competitively mediates for a left ear response, this creates a cognitive conflict situation (for further details, see Hugdahl et al. 2009).

We now follow-up on the fMRI findings from van Wagningen et al. (2009) to validate the findings with a magnetic resonance spectroscopy (MRS) study, with the hypothesis that the reduced neuronal activation found in van Wagningen et al. (2009), when participants were instructed to pay attention only to the left ear stimulus after memantine administration, is mediated at the receptor level through reduction in glutamate and glutamine (Glx) levels in the frontal lobe. To our knowledge, there are no proton $^1$H-MRS studies that have investigated the effects of memantine on the glutamatergic system in healthy individuals and related them to fMRI results. $^1$H-MRS is a noninvasive sampling method that can be used to measure metabolites in the brain, including glutamate. Previous studies using $^1$H-MRS (Glodzik et al. 2008) have shown that administration of memantine for 6 months to Alzheimer’s patients resulted in a reduction of glutamate concentration in hippocampus. The efficacy of memantine in reducing glutamate receptor site concentrations is due to the low affinity for and rapid voltage-dependent interaction with the NMDA receptor. Because the resonance peaks of Glx could not be reliably separated at field strength of 3 T, the current study investigated the effect of memantine on the combined Glx concentration in healthy individuals with the hypothesis that memantine administration would lead to a decrease in Glx concentrations particularly in areas related to cognitive control functions. Moreover, Glx concentrations were correlated with blood oxygen level-dependent fMRI data for both the drug-naive and memantine sessions. Twenty-five healthy individuals were scanned twice in a counterbalanced design, drug naïve, and after 21 days with memantine administration.

**Materials and Methods**

**Participants**

The participants were 25 right-handed healthy adults, 14 males and 11 females (mean age = 23.7 years, standard deviation [SD] = 3.83), mean body weight 71.70 kg (SD = 11.67, n = 23). Handedness was determined from a 15-item handedness questionnaire developed by Raczkowski et al. (1974) and in order to be included in the study at least 13 of the 15 items had to be checked for right hand (or right foot, 1 item) use. The participants received written and oral information about the project, the procedure at the laboratory, and about memantine, prior to inclusion in the study. These included avoidance of nicotine and caffeine for at least 2 h prior to the MR scanning. All participants gave written informed consent and were paid a compensation for their expenses and use of time. The study protocol was approved by the Norwegian Medicines Agency (EUDRACTN. 2005-002610-26), the Norwegian Social Science Data Service, and the Regional Ethics Committee for Medical Research (REK) and was carried out according to the declaration of Helsinki. In addition, all participants were insured by the Drug Liability Association (Act 23.12.88 nr.104).

The participants were screened with audiometric testing for the frequencies of 500, 1000, 2000 and 4000 Hz to ensure normal hearing on both ears. Participants with a threshold higher than 20 dB on any frequency, or an interaural difference larger than 15 dB, were excluded from the study (no participants were excluded).

**Memantine Administration and Procedure**

Memantine (Ebixia), H. Lundbeck A/S (EU/1/02/219/002/NO), was used for the pharmacological intervention. Memantine is generally well tolerated (Arcosa et al. 2005), and the most frequently occurring adverse events in patients taking memantine (<1%) include: confusion, dizziness, drowsiness, headache, insomnia, agitation, and/or hallucinations. Less common adverse effects (<1%) include: vomiting, anxiety, hypertonia, cystitis, and increased libido (Rossi 2006). None of the participants reported any severe side effects. About half the participants reported slight tiredness and dizziness the first few days of memantine administration, and in a few instances, this was also reported at the highest dose (20 mg). For 2 participants, the dizziness was combined with slight nausea. No participants experienced any serious adverse events occurred during the study or during the initial 30 days following the study; as determined by personal contact with a psychiatrist (H.A.J.) who was responsible for the drug administration and continuous follow-up. Memantine was administered orally as tablets to be taken every day for 21 days. In order to reduce the risk of side effects, the dose of memantine was increased weekly from 10-15 to 20 mg/day to obtain close to steady-state plasma level after 21 days which is reported from clinical trials to remain in the order of 0.5–1 μmol (Parsons et al. 1999). A within-subject design was used and the participants were scanned twice, drug naïve, and after 21 days on memantine. Because the participants performed the dichotic listening task on several occasions, this might create carry-over effects, therefore changes may not be related to the effect of interest, for example, the effects of memantine, but rather be an effect of learning or repetition. This was, however, controlled for by using a counterbalanced design where half of the participants were first tested drug naïve and the other half first tested after memantine administration and thereafter tested with an analysis of variance (ANOVA) with repeated measures, significance threshold set to $P < 0.05$.

**Auditory Task (Dichotic Listening)**

The dichotic listening task was presented under 3 different instruction conditions; no focused attention, attention focused on the right ear stimulus, and attention focused on the left ear stimulus (Hugdahl and Andersson 1986). However, since no effects of memantine were seen in the no-instruction, and right ear instruction condition, we only present data for the left ear instruction condition (van Wageningen et al. 2009), which also is the condition tapping cognitive control processes. There were 30 stimulus presentations for each condition, each presentation lasting about 500 ms and with 3.5-s interstimulus intervals. The consonant-vowel syllables used were /ba/, /da/, /ga/, /pa/, /ka/, and /ta/, with all possible pair-wise combinations, excluding the homonyms. The syllables were read by an adult voice and they were digitally recorded, stored, and presented through MR-compatible headphones (NordicNeuroLab Inc., Bergen, Norway, www.nordicneurolab.com) using E-prime software (www.pstnet.com). The headphones also served to attenuate background noise (~40 dB) from the MR magnet gradients. The participants responded verbally on each trial through an in-house built air-conducting microphone, placed on the head coil, and connected to a digital recorder (M-Audio Microtracker 24/96, www.m-audio.com) outside the MR chamber.
MR Scanning
MR scanning was performed with a 3.0-T GE Signa HDx scanner, using a single-channel head coil. Head movements were restrained by supportive padding, inside of the head coil. For positioning the slices for functional imaging parallel to the AC-PC line, a high-resolution $T^*_2$-weighted image was acquired prior to the echo planar images (EPI). In addition, an axial $T^*_2$-weighted image was acquired prior to the $^1$H-MRS imaging sequences.

$^1$H-MRS Scanning and Data Analysis
In vivo short echo $^1$H-spectra from the right and left frontal cortex were obtained by using a single-voxel point-resolved spectroscopy (PRESS) sequence (voxel size $20 \times 20 \times 20$ mm$^3$, time repetition/time echo [TE] = 1500/35 ms, 128 averages) for the MRS measurements. See Figure 1 for the localization of the left and right voxel. PRESS was chosen because this gives a high signal to noise ratio that may be crucial for the unambiguous detection of minor signals and improved accuracy of metabolite quantification. The shimming procedure was optimized to get a water suppression level of at least 96%. The line width was on average for the drug-naive session 9.4 (SD = 1.47) for the left frontal spectra and 9.72 (SD = 1.24) for the right frontal spectra, and for the memantine session, the line width was on average 9.64 (SD = 2.0) for the left frontal spectra and 10.16 (SD = 1.62) for the right frontal spectra. Single voxel spectra were analyzed using LC model software (LCModel Version 6.1-4E [Provencher 1993]), allowing metabolite concentrations to be calculated. Due to possible ambiguity in differentiating the Glx resonance at 3-T magnetic field strength, the combined resonance Glx concentration is reported. The N-acetyl-aspartate (NAA) and creatine concentrations were also measured in order to validate specific changes in concentration. The NAA and creatine resonance at 3-T magnetic field strength, the combined resonance Glx concentration is reported. Due to possible ambiguity in differentiating the Glx resonance at 3-T magnetic field strength, the combined resonance Glx concentration is reported. The N-acetyl-aspartate (NAA) and creatine concentrations were also measured in order to validate specific changes in concentration.

fMRI Scanning and Data Analysis
For the functional imaging session, a sparse-sampling EPI acquisition scheme (Hall et al. 1999) was selected (64 × 64 matrix, 25 slices, 5-mm slice thickness, 0.5-mm gap, TE 30 ms, 4 dummy scans, 180 volumes), with an acquisition time of 1.5 s, followed by a silent gap of 4 s for stimulus presentation and verbal response recording (van den Noort et al. 2007). The experimental design was a single session block design with 9 blocks, 3 per condition, starting with 3 blocks with the no attention instruction condition, followed by the conditions where attention was focused on the right ear stimulus and left ear stimulus in a pseudorandomized order. Each block contained 10 trials, with one trial per silent gap. Each block of trials was followed by a 55-s rest condition without stimulation.

fMRI Data
FL Condition
A 2-sample $t$-test analysis for the difference contrast of drug-naive versus memantine conditions showed a significant cluster in left medial frontal gyrus ($x = -12, y = 48, z = 24, t = 4.31$), which exceeded a small-volume corrected threshold of (family wise error) $P < 0.05$ and contained at least 10 voxels. The reversed contrast, memantine versus drug-naive session did not yield any significant activation.

MRS Data
An ANOVA with repeated measures showed a significant reduction of Glx concentration in the right frontal spectra $F_{1,24} = 9.0875, P = 0.006$ after memantine administration compared with when the participants were drug naive, see Table 1.

Table 1
The table shows the descriptive statistics for the behavioral dichotic listening data for the 3 attention conditions

<table>
<thead>
<tr>
<th>Mean</th>
<th>SD</th>
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<tbody>
<tr>
<td>Drug naive</td>
<td>%RE</td>
</tr>
<tr>
<td>NF</td>
<td>47</td>
</tr>
<tr>
<td>FR</td>
<td>60</td>
</tr>
<tr>
<td>FL</td>
<td>39</td>
</tr>
<tr>
<td>Memantine</td>
<td>%RE</td>
</tr>
<tr>
<td>NF</td>
<td>46</td>
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<tr>
<td>FR</td>
<td>56</td>
</tr>
<tr>
<td>FL</td>
<td>39</td>
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Note: %RE, % of correct reports from the right ear; %LE = % of correct reports from the left ear; LI = laterality index: [(%RE - %LE)/(%RE + %LE)] × 100.
Table 2. There was no significant difference in Glx concentration for the left frontal spectra after memantine administration compared with when the participants were drug naive. There were no significant differences in NAA and creatine concentrations for the left frontal and right frontal spectra after memantine administration compared with when the participants were drug naive, see Table 2.

**Correlation of Glx Concentrations and fMRI Data**

The difference contrast between the drug-naive versus memantine sessions showed that significant decrease in Glx concentrations, both from the right and left frontal spectra, were associated with reduction in activation in frontal cortex for the left ear instruction condition, after memantine administration. In more detail, using the Glx concentration, measured in the right frontal cortex as covariate, the small-volume corrected analysis for the difference contrast of drug-naive versus memantine sessions demonstrated a significant correlation in the left middle frontal gyrus ($x = -24$, $y = 54$, $z = 15$, $t = 4.17$), see Figure 2. Using the Glx concentration, measured in the left frontal cortex, the small-volume corrected analysis for the same contrast showed a significant correlation with an area in left medial frontal gyrus ($x = -15$, $y = 57$, $z = 12$, $t = 4.11$) and for the right medial frontal gyrus ($x = 6$, $y = 63$, $z = 9$, $t = 3.90$), see Figure 2. All reported areas exceeded a small-volume corrected threshold of (FDR) $P < 0.05$ and contained at least 10 voxel.

**Effects of Order**

In order to validate that there were no carry-over effects in this counterbalanced design, an ANOVA with repeated measures was estimated, revealing that there were no significant order effects in the MRS and fMRI data, when significance threshold was set to $P < 0.05$.

**Discussion**

The results showed a specific decrease of Glx concentration after memantine administration in the right frontal spectrum. There were no significant differences in NAA and creatine concentrations for the left frontal and right frontal spectra after memantine administration compared with when the participants were drug naive. The fMRI findings for the FL condition resulted in a reduction in activation in the left medial frontal gyrus after memantine administration compared with when the participants were drug naive. Moreover, it was also shown that the difference contrasts for the FR condition for the drug naive versus memantine sessions did not yield any significant activation (van Wageningen et al. 2009). It has been shown that PFC and ACC activations are typically observed with tasks involving requirements for cognitive control (Duncan and Owen 2000; Landro et al. 2001; Stuss 2006). The FL and FR condition both involve top-down attention instruction modulation. However, in the FL condition there is in addition a processing conflict in the sense that the bottom-up effect will favor a right ear response, whereas the top-down effect will favor a left ear response. Thus, the FL situation also involves a cognitive control or cognitive conflict situation. This situation requires cognitive control in the form of suppression of the bottom-up stimulus-driven effect. Although, in the FR situation, the 2 processes act synergistically, both pushing for a right ear response. Hugdahl et al. (2009) have suggested that instruction to focus attention on the right or left ear stimulus induces different degrees of cognitive conflict and the corresponding need for cognitive control. This has been supported by neuroimaging results by Thomsen et al. (2004) when using the same auditory task, they showed that the FL condition activated PFC and dorsolateral ACC (see also Jancke and Shah 2001) who found PFC activation in a similar task. The results showed that memantine significantly reduced Glx concentration and PFC activation, suggesting an effect on cognitive control functions.

Furthermore, when Glx concentrations were correlated with fMRI data from the FL condition, the results showed that reduced Glx concentrations were associated with reduced activation in PFC, after memantine administration. This association was observed both for the left and right frontal spectra and would be expected from the key role played by glutamate for neuronal excitation and hence for increase in neuronal activation. Thus, the current findings indicate that the reduced neuronal activation in relation to cognitive control functions, after memantine administration, can be explained by a reduction in glutamatergic neurotransmission, measured as a reduction of Glx concentration in frontal spectra.

By using Glx concentrations as a measure for glutamatergic neurotransmission, the effect of memantine on overall glutamatergic function was measured. In excitatory neurotransmission, glutamate is released into the synaptic cleft from the neurons where it binds to receptors at postsynaptic neurons. Glutamate is rapidly removed from the synaptic cleft by uptake into the astrocytes where it is converted into glutamine and then transported back to the presynaptic neuron for reconversion to glutamate. The Glx cycle is thought to be an important pathway by which glutamate is recycled after release.

Figure 2. The difference contrast between the drug-naive versus the memantine sessions showed that significant decrease in Glx concentration, both from the (a) left and (b) right frontal spectra, were associated with reduction in activation in frontal cortex for the left ear instruction condition, after memantine administration (axial slice position at 11 mm in Montreal Neurological Institute space, the image on the left shows the correlation for the left frontal spectra, and the image on the right shows the correlation for the right frontal spectra).
(Magistretti and Pellerin 1999; Novotny et al. 2003). Thus, this cycle is critical to maintain proper regulation of glutamatergic neurotransmission and therefore is important for many brain functions. Glodzik et al. (2008) suggested that repeated administration of NMDA antagonists could change the total concentration of glutamate. Decreased glutamate release and/or impaired astrocyte function would be expected to result in decreased glutamate flux through the Glx cycle. Rothman et al. (2003) have proposed that the physiological responses observed in fMRI that arise from increased energy demand with neuronal activation are directly related to the Glx cycle (Rothman et al. 2003). Thus, the current results show that the glutamatergic system seems to be sensitive to pharmacological manipulation, and the results suggest that the regulation of the system is decreased after repeated memantine administration affecting brain activation related to cognitive control functions.

Previous studies, using the dichotic listening paradigm by Hugdahl and Andersson (1986), have shown that both schizophrenia patients and elderly individuals have reduced abilities in the top-down modulation when instructed to focus on and only report the left ear stimulus, as in the FL condition (Loberg et al. 1999; Andersson et al. 2008). These findings are further supported by studies showing that schizophrenia patients are impaired in tasks requiring top-down cognitive control and response suppression (Green 1998; Heinrichs 2000; Rund et al. 2006). One possible explanation is that this may be due to altered glutamatergic neurotransmission in schizophrenia patients, which is supported by findings showing lower levels of glutamatergic metabolites in ACC and PFC in chronic patients compared with healthy controls (Tsai et al. 1995; Theberge et al. 2003). It has been demonstrated that first time drug-naïve schizophrenia patients have elevated glutamine levels, which is a putative marker of glutamate neurotransmitter release (see, however, Theberge et al. 2002). Moreover, it has also been demonstrated that acute ketamine administration increases blood flow in frontal and cingulate cortex in healthy individuals (Holcomb et al. 2005). In contrast, Glodzik et al. (2008) showed that chronic administration of memantine, for 6 months, to a group of mild cognitive impairment and Alzheimer’s disease resulted in decreased glutamate levels in hippocampus. Findings from the current study are in line with the findings from Glodzik et al. (2008), suggesting that repeated administration of memantine results in decreased glutamatergic neurotransmission. The present results therefore suggest that chronic or repeated administration of NMDA receptor antagonists modulates NMDA receptor function and decreases glutamatergic neurotransmission, whereas an acute dose has been shown to increase Glx concentrations (Glodzik et al. 2008). We have previously shown that there were no significant effects on behavioral performance between the drug-naïve and memantine sessions for the FL condition (van Wageningen et al. 2009). The absence of significant effects on behavioral performance has the advantage that differences in the activation pattern cannot be explained as the result of performance differences between the 2 imaging sessions, which might confound the interpretation of the neuronal effects of the drug because variation in brain activation may reflect differences in task performance rather than revealing an effect of the condition of interest, the effect of the drug (Fu and McGuire 1999).

It is concluded that the present findings provide evidence that glutamate may be mediating cognitive control at the receptor/transmitter level, as seen in the FL instruction condition. A glutamatergic effect for the FL findings in the forced-attention paradigm has not previously been demonstrated and could have implications for the understanding of the neurophysiological underpinnings of higher cognitive functions, like cognitive control and for PFC functioning.

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